Modeling

Overview

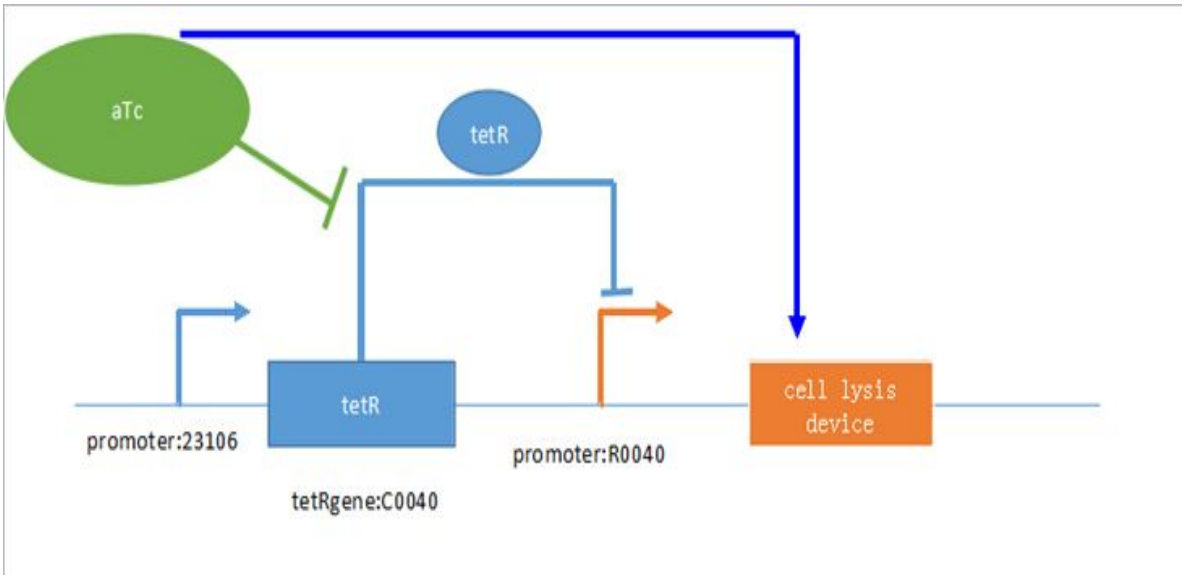
In this part,we analysed the chain suicide loop of cell lysis and establish the mathematical model,which is divided into the following three parts.First,mechanism analysis is to analyse the mechanism of the relationships among anhydrotetracycline(aTc), tet protein and cracked cells in suicide loop.Second, through Modeling and solving we identified the reasonable aTc concentration range, and established the ODE models according to the reaction mechanism,eventually got the relationship of cell lysis , aTc concentration and the time.We also analysed the experimental results using L-arabinose instead of aTc.Last,we gave 2 promotions of the model,which respectively are the general mathematical description of the chain loop and the new circuit regulated by quorum principle.

Mechanism analysis

Background

In order to make both TAT: : H4 fusion protein produced by the engineering bacterium and the transfected plasmid to be released in full,we designed corresponding suicide gene in the transfected plasmid,which has to express at the appropriate time. We used chain suicide loop in our project,which is simple and direct.However, the aTc itself as a kind of antibiotics will affect the cell itself in the process of adding, and thus make factors complicated to affect the reaction result, which is not conducive to the analysis of the model.

Introduction of the suicide loop



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On the one hand,tetR protein itself can repress the promoter R0040, that is to say,under normal conditions R0040 promoter is repressed and cell lysis does not happen.But the gene expression of tetR protein is restrained when people add the aTc , thus the number of tetR proteins in the cells will fall,and the repression of promoter R0040 respective will drop,leading cell lysis device to start and the cells to crack.On the other hand,aTc itself also can lead to cell lysis which brings difficulties to the analysis of the experimental result. As a result,we should first identify the aTc concentration range to ensure aTc itself make less effect on cells, and then we can do quantitative analysis of the experimental process within this range.

Two main problems

According to the analysis above, we put forward the following two major problems to solve: Problem 1: We need to identify a reasonable aTc concentration range to make aTc itself have less effect on the engineering bacteria but also can effectively induce the expression of the lysis gene. problem 2: We need to establish a reasonable ODE model to describe the relations of each material in the process of lysis and to guide the biological experiments.

Modeling and solving

Model assumes

assume 1: There is only one factor that influences the suicide process in the experiment without any other affection.

assume 2: There is only one process of the chain reaction without other bifurcate structures.

Symbol Description

symbols	meaning
$[tetR_{protein}]$	Concentration of tetR protein
[aTc]	Concentration of aTc
sp	the amount of cracked cells
K_1 K_2	Coefficients in ODE
α_1 α_2	
β	
C_1 C_2	

Problem 1:To identify the aTc concentration range

In order to find out reasonable aTc concentration range to make sure the cell death is mainly associated with the suicide mechanism.Therefore we designed a set of control experiment to To detect the reasonable aTc concentration.

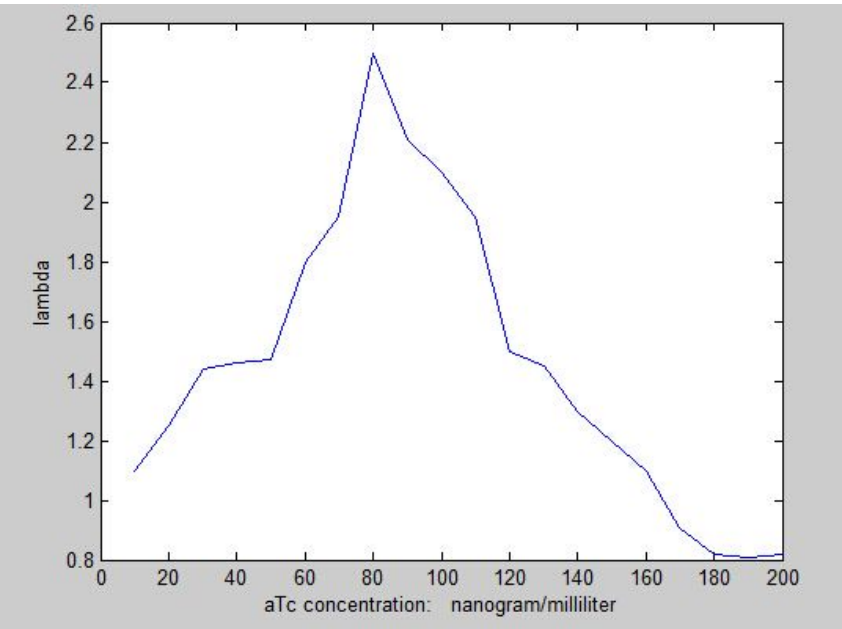
We suppose there are two groups of E.coli cells with similar cell activity (A and B).Engineered E. coli cells of group A contained artificial plasmid,and the aTc can trigger the cell lysis.While another E. coli cells of group B does not contain artificial plasmid,whose growth directly affected by aTc itself.According to assume 1, there is only one factor that influences the suicide process in the experiment without any other affection.Therefore E.coli cells of group B will be directly affected by aTc and result in cell lysis, while engineered E.coli cells of group A will be affected by both aTc and cell lysis.

In order to accurately determine the suitable aTc concentration,we introduced a quantity λ ,which is the ratio of numbers of cells of group B and group A per unit time under different aTc concentrations:

$$\lambda = \frac{num_b}{num_a}$$

The formulas shows that the main reason of cell death can be attributed to the influence of suicide system when λ is larger .

So far, we have presented a set of method to detect reasonable aTc concentration range.According to the results UCB has given in 2012 iGEM,it's better when aTc concentration range is between 0 and 140 nanogram per milliliter.The numerical simulation diagram is given.



We carried out on the numerical fitting by using this result and got the figure :

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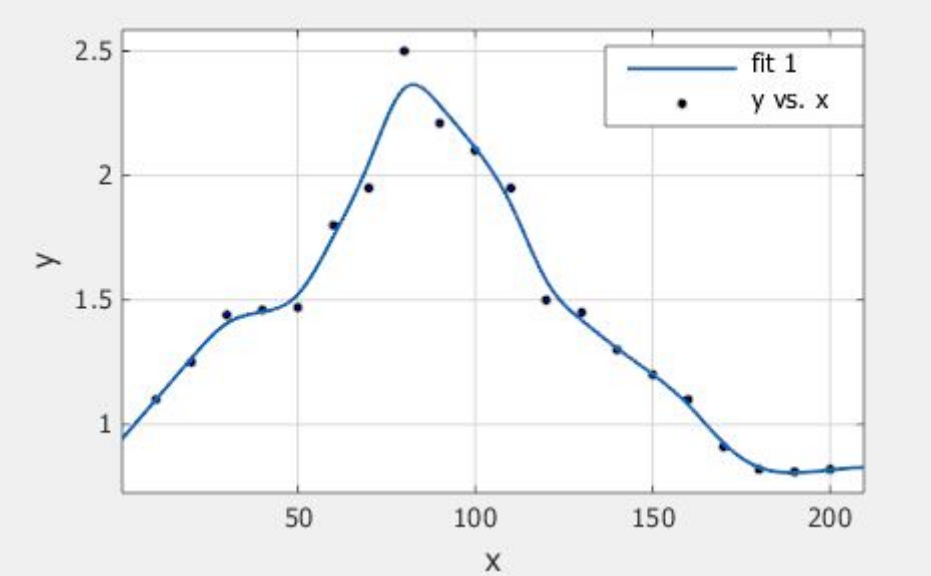


Thus we can analyse and conclude that, λ is larger when the aTc concentration is from 80 to 110 grams per milliliter, at the same time,it illustrates the suicide mechanism we designed works well when the aTc concentration is in this range. That is to say, it can be more powerful to kill cells and control numbers of cells.

Problem 2：ODE Model

aTc inhibits the expression of tetR gene,so that in the part of the tetR protein producing, we deduced that the productive rate of the tetR protein is inversely proportional to aTc concentration,and got the equation.

$$\frac{d[tetR_{protein}]}{dt} = K_1 \frac{1}{[\mathrm{aTc}]} + B$$

At the same time, tetR protein also inhibits its production,therefore we can identify:

$$B = -\alpha_1[tetR_{protein}]+\alpha_2$$

Combined with the two equations we got:

$$\frac{d[tetR_{protein}]}{dt} = K_1 \frac{1}{[\mathrm{aTc}]} - \alpha_1[tetR_{protein}] + \alpha_2 \qquad (1)$$

TetR protein produced by the first step in the chain reaction can inhibit the promoter of lysis unit to express,so we described the relation between the rate of lysis and tetR protein concentration in terms of the equation:

$$\frac{d[sp]}{dt} = K_2 \frac{1}{[tetR_{protein}]^n} + C$$

sp is the amount of the cracked cells, n is the reaction order of the combination between tetR

protein and the lysis device which need obtaining by the experiment. C as an unknown variable, needs to be discussed below.Because aTc itself can lead to cell lysis,we determined that C is a function of aTc concentration and can stimulate cell lysis, so we concluded that:

$$C = \beta[\mathrm{aTc}]$$

So in the part of cell lysis,we set up the following equation to describe the reaction of suicide loop:

$$\frac{d[sp]}{dt} = K_2 \frac{1}{[tetR_{protein}]^n} + \beta[\mathrm{aTc}] \tag{2}$$

Simultaneous equations above to establish system of equations:

$$\begin{cases} \dfrac{d[tetR_{protein}]}{dt} = K_1 \dfrac{1}{[\mathrm{aTc}]} - \alpha_1[tetR_{protein}] + \alpha_2 \\ \dfrac{d[sp]}{dt} = K_2 \dfrac{1}{[tetR_{protein}]^n} + \beta[\mathrm{aTc}] \end{cases} \tag{3}$$

System of equations (3) can describe the reaction mechanism of the suicide system. First we solved equation (1),and obtained the relation between the tetR protein concentration under the different aTc concentration and the time:

$$[tetR_{protein}] = \frac{K_1 + \alpha_1[\mathrm{aTc}] + C_1 e^{-\frac{\alpha_2}{t}}}{\alpha_2[\mathrm{aTc}]} \tag{4}$$

Among this α_2 controls the initial reaction rate,and C_1 is an undetermined constant.We substituted equation (4) into equation (2) to solve the differential equation, and obtained the function of the amount of cracked cells :

$$sp = C_2 + \frac{t\,(K_2 + \beta[\mathrm{aTc}]\,[tetR_{protein}]^n)}{[tetR_{protein}]^n} \tag{5}$$

We can carry on the numerical simulation By (4) (5) .In order to calculate conveniently we

set $K_1 = \alpha_1 = \alpha_2 = 1, C_1 = 20$,and drew a diagram about the relation between the tetR protein concentration under the different aTc concentration and the time,which is as shown in the figure 1:

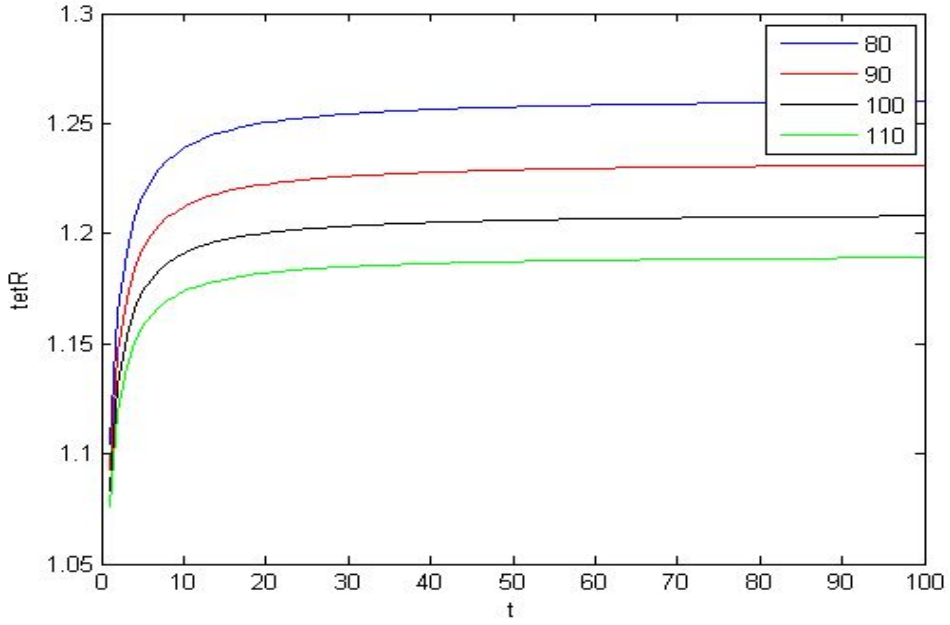


Fig 1

From the diagram above we can see that the tetR concentration falls as aTc concentration rises in the range of 80 to 110 .We can further analyse the variation law of the number of cracked cells with time under different aTc concentration in the case that the tetR concentration is known.As well,in order to calculate conveniently, we set $K_2 = \beta = 1$ and drew a diagram about it,which is as shown in figure 2:

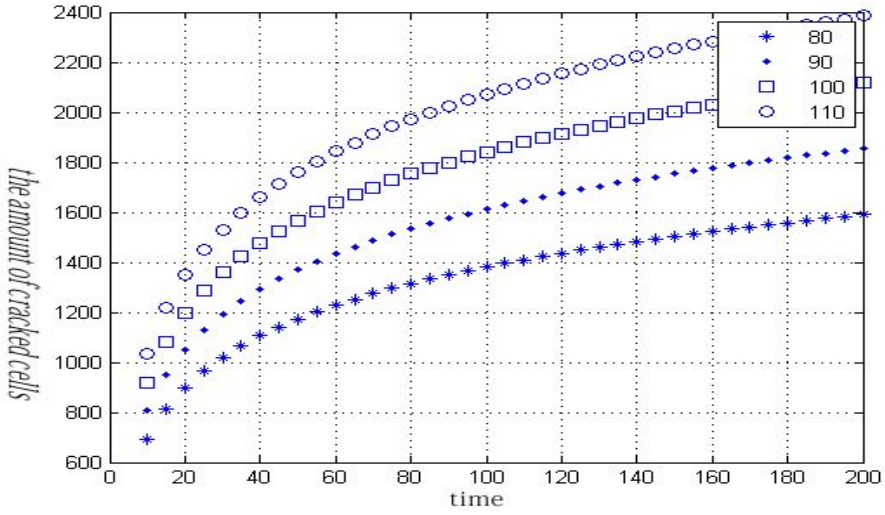


Fig 2

The diagram above shows that the amount of the cracked engineering bacterium cells increases with time grows and the trend becomes smooth.It also increases with the aTc concentration increases.

To sum up, we have completed the quantitative analysis in every process of suicide system, and given the mathematical description of the product and changes in the biological reaction process. We also obtained the reasonable scope of aTc concentration

and the number of cracked cells with time under different aTc concentration,and thus completed the establishment and solution of the model.

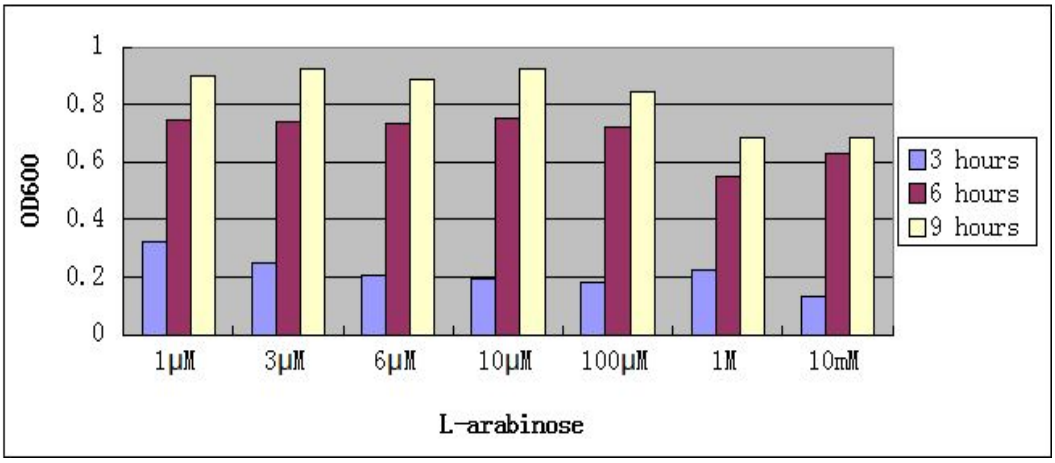
Through the model we can theoretically give the mathematic expression of the aTc concentration and the amount of cracked cells, but the coefficients need to be determined in the subsequent experiments,after which we can estimate the amount of cracked cells via the aTc additive amount to predict the results of experiments.And we can also control the aTc additive amount via the amount of cracked cells as a threshold value.

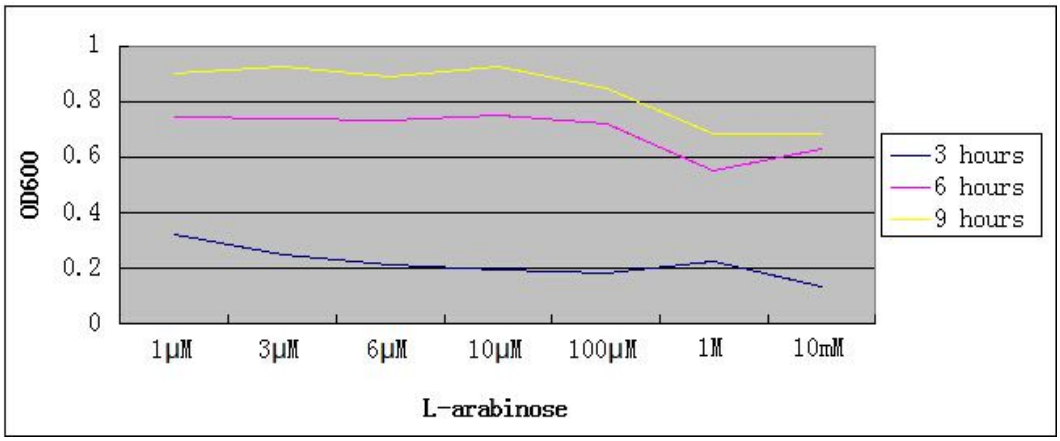
Analysis of the experimental results using L-arabinose instead of aTc

We used L-arabinose to replace aTc and then conducted the experiment.L-arabinose can induce lysis device but not kill cells directly.Experimental datas are as follows:

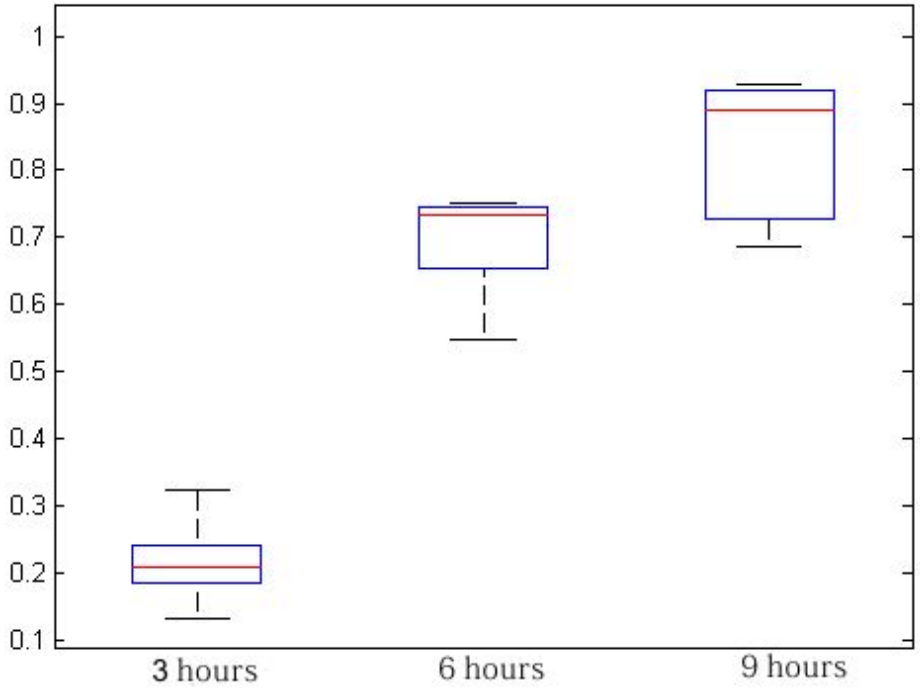
L-arabinose	3 hours	6 hours	9 hours
1μM	0.322	0.745	0.901
3μM	0.247	0.741	0.927
6μM	0.209	0.733	0.891
10μM	0.191	0.75	0.924
100μM	0.183	0.721	0.848
1M	0.222	0.548	0.687
10mM	0.132	0.631	0.685

According to the data we drew the bar chart and the line chart:





In order to demonstrate the reliability of the experiment, we used one-way analysis of variance to analyse the experiment above and got the diagram below:



Through one-way analysis of variance about the OD600 after 3 hours, 6 hours and 9 hours, we can see that the addition of L-arabinose significantly induced the cell lysis and the effect is remarkable.Thus we got the conclusion that L-arabinose can lead to cell lysis and have a good experimental effect.

Promotion

The analysis of the model

The model can well reflect the suicide mechanism and The effects of aTc content on the cell cracking,which has a certain practical value.At the same time,it can be concluded as a method suitable for more experiments,and complete the quantitative description of the experiment.However,the model is relatively simple,only considering the effects of the single variable on the cells, but in reality, the activities of the cell and the changes of its number not only controlled by one variable, and it is not given a better strategy of the random interference factors in the experiment. So it need improving in the future research.

Promotions of the model

Promotion 1:

On the basis of the experiment, we found that experiments which meet the topological structure shown in figure 3 can take advantage of this model to describe.

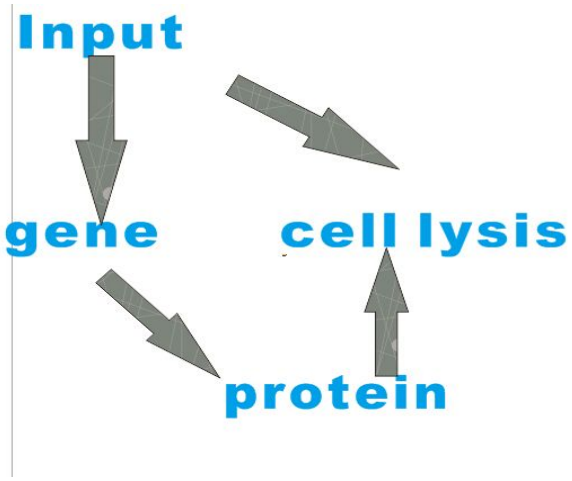


Fig 3

Input is a single variable which has influence on cells,and it can promote the expression of a specific purpose gene to produce the protein which can make cell lysis.At the same time,many kinds of inputs are antibiotic substances, therefore the input itself can cause cracking of cells directly.For such experiments it can be described by the equation:

$$\begin{cases} \dfrac{d[protein]}{dt} = K_1 \dfrac{1}{[\mathrm{input}]} - \alpha_1[protein] + \alpha_2 \\ \dfrac{d[\mathrm{crack}]}{dt} = K_2 \dfrac{1}{[protein]^n} + \beta[\mathrm{input}] \end{cases}$$

Thus we can accomplish to solve a class of problems and promote the models.

Promotion 2:

On this basis, we further promoted the system regulated by quorum sensing principle,which makes the new suicide circuit work better.

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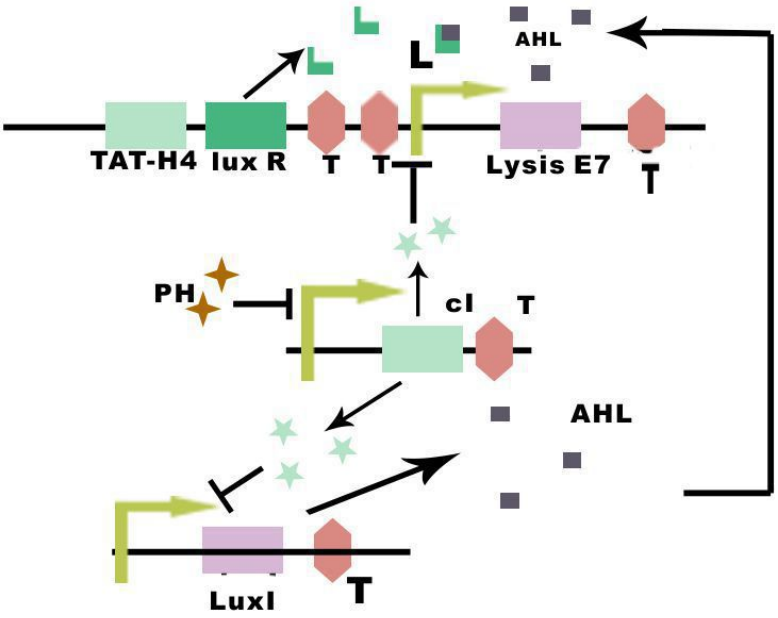
Symbol Description

symbols	meaning
[c]	Concentration of cI protein
input	PH
[l]	Concentration of Lux protein and AHL protein binding
[lux]	Concentration of Lux protein
$\alpha_1, \alpha_2, \alpha_3$	The equilibrium constant
$\beta_1, \beta_2, \beta_3$	
$k_1\ k_c\ k_{c1}\ k_{c2}$	Synthesis rate
n,m,p,q	The order of reaction

The following response structure is given:

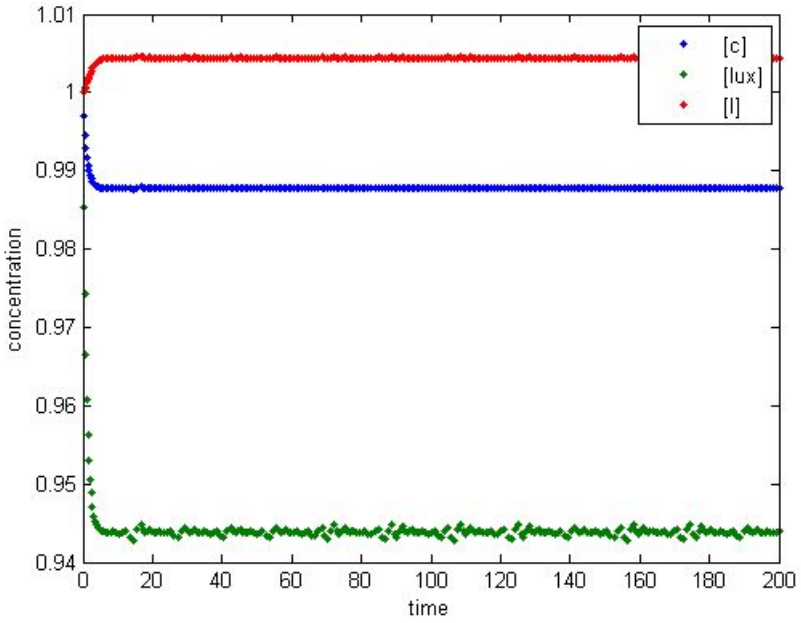


This system can better control lysis compared with the chain reaction before,and we gave the ODE model to describe the reactions:

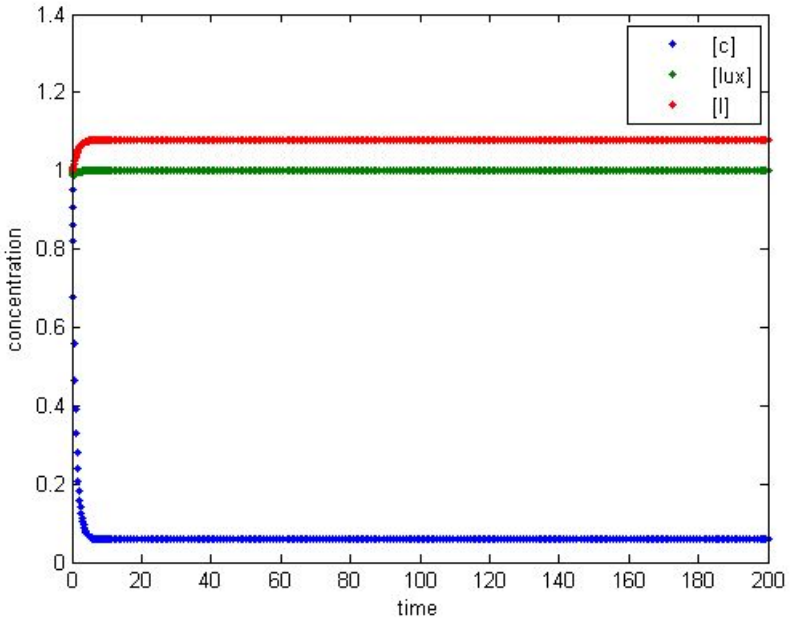
$$\begin{cases} \dfrac{d[\mathrm{c}]}{dt} = \dfrac{\beta_3[\mathrm{input}]^n}{k_1{}^n + [\mathrm{input}]^n} - \alpha_3[\mathrm{c}] \\ \dfrac{d[\mathrm{l}]}{dt} = \dfrac{\beta_1 k_{c1}{}^m}{k_{c1}{}^m + [\mathrm{c}]^m} + \dfrac{\beta_2[\mathrm{lux}]^p}{k_c{}^p + [\mathrm{lux}]^p} - \alpha_1[\mathrm{l}] \\ \dfrac{d[\mathrm{lux}]}{dt} = \dfrac{\beta_3 k_{c2}{}^q}{k_{c2}{}^q + [\mathrm{c}]^q} - \alpha_2[\mathrm{lux}] \end{cases}$$

We used numerical method to solve the equations. According to the experience,we set n=m=p=q=4, $\alpha_1=\alpha_2=\alpha_3=\beta_1=\beta_2=\beta_3$=2, $k_1=k_c=k_{c1}=k_{c2}$=1.

Diagram is as follows when PH=10:

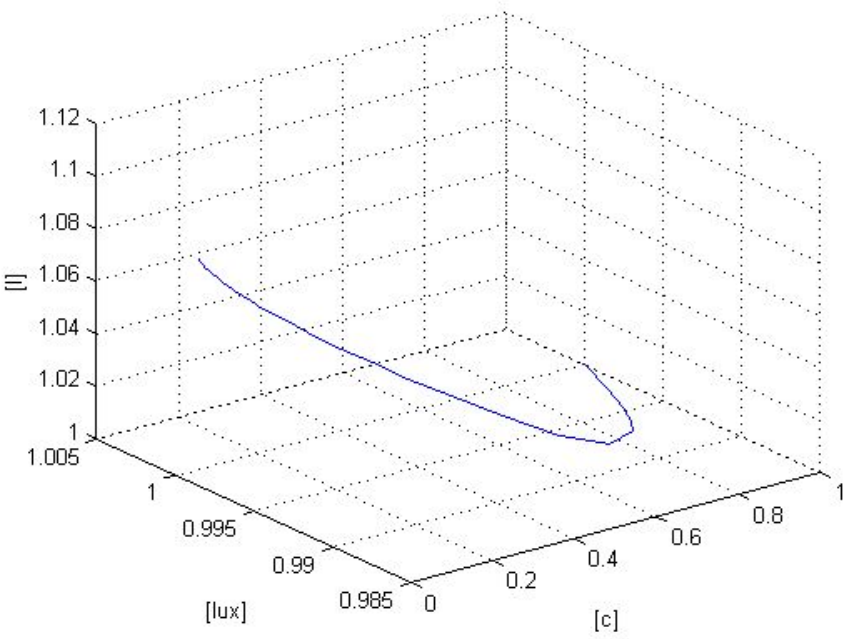


We set PH = 1 under the premise of other coefficients invariant staying constant and got the diagram below:

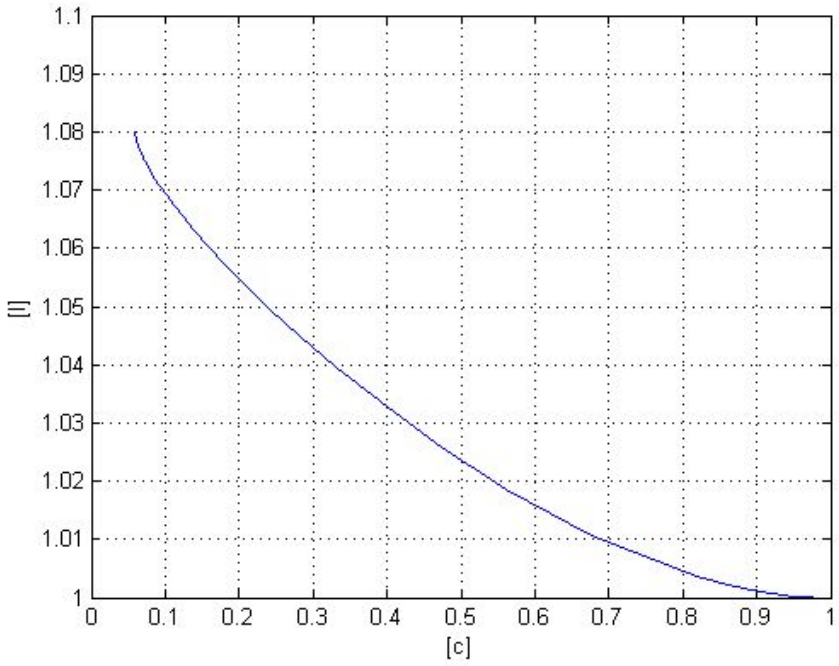


Thus we can see that PH can effectively affect the content of three kinds of variables , and then control cell lysis.

In oder to reflect the interaction of three kinds of variables intuitivly,we can draw the diagram among the three,which is as follows (PH = 1)



And we can also drew the diagram of any two variables: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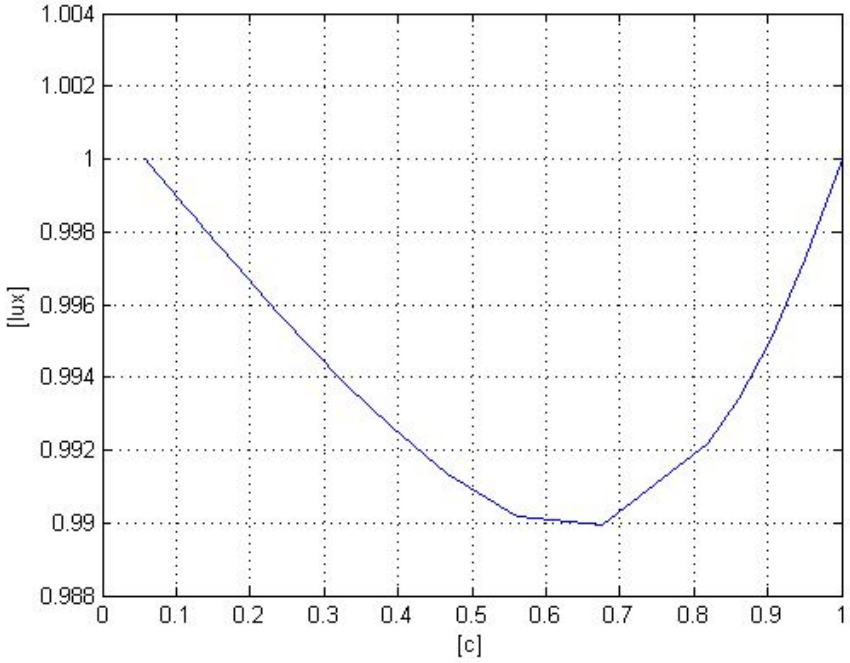
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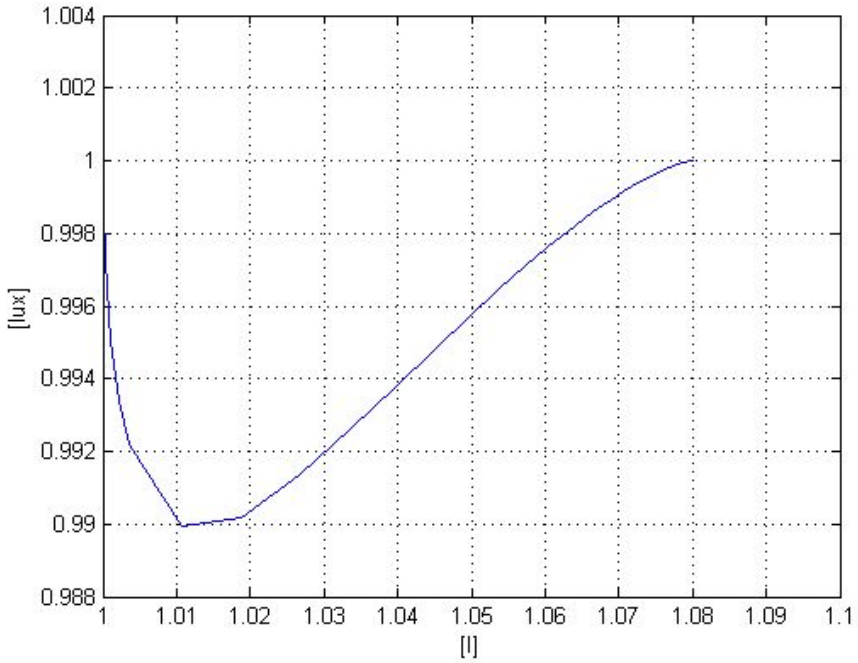

References

[1] Thomas Lederer,Martin Kintrup,Masayuki Takahashi, Phaik-Eng Sum,George A. Ellestad,and Wolfgang Hillen, Tetracycline Analogs Affecting Binding to Tn10-Encoded Tet Repressor Trigger the Same Mechanism of Induction,Biochemistry 1996, 35, 7439-7446.

[2] Casonya M. Johnson and Robert F. Schleif,In Vivo Induction Kinetics of the Arabinose Promoters in Escherichia coli,Journal Of Bacteriology, June 1995, p. 3438–3442.